

Amendments to the claims:

Please cancel claims 1-50 without prejudice or disclaimer to their renewal in a subsequently filed application, and enter the following new claims as indicated below.

This listing of claims will replace all prior versions, and listings, of claims in the application:

51. (new) A method of creating, in a eukaryotic cell, a modified endogenous gene locus flanked downstream by a site-specific recombination site comprising:

- (a) creating a large targeting vector for use in eukaryotic cells (LTVEC) comprising a site-specific recombination site, a downstream homology arm containing a region homologous to a 3' end of the endogenous gene locus region and an upstream homology arm within the locus;
- (b) introducing the LTVEC of (a) into a eukaryotic cell; and
- (c) using a quantitative assay to detect modification of allele (MOA) in the endogenous gene locus to identify those eukaryotic cells in (b) in which the endogenous gene locus is flanked downstream by the site-specific recombination site.

52. (new) A method of creating, in a eukaryotic cell, a modified endogenous gene locus flanked upstream by a site-specific recombination site comprising:

- (a) creating a large targeting vector for use in eukaryotic cells (LTVEC) comprising a site-specific recombination site, an upstream homology arm containing a region homologous to the 5' end of the endogenous gene locus region and a downstream homology arm within the locus;
- (b) introducing the LTVEC of (a) into the eukaryotic cell; and
- (c) using a quantitative assay to detect modification of allele (MOA) in the endogenous gene locus to identify those eukaryotic cells in (b) in which the endogenous gene locus is flanked upstream by the site-specific recombination site.

53. (new) A method of creating, in a eukaryotic cell, a modified endogenous gene locus flanked by site-specific recombination sites comprising:

- (a) creating a first large targeting vector for use in eukaryotic cells (LTVEC) comprising the site-specific recombination site, a downstream homology arm containing a region homologous to the 3' end of the endogenous gene locus region and an upstream homology arm within the locus;
- (b) creating a second LTVEC comprising the site-specific recombination site, an upstream homology arm containing a region that flanks the 5' end of the endogenous gene locus region and a downstream homology arm within the locus;
- (c) introducing the first and second LTVECs into the eukaryotic cell; and
- (d) using a quantitative assay to detect modification of allele (MOA) in the endogenous gene locus to identify eukaryotic cells in which the site-specific recombination sites are flanking the endogenous gene locus.

54. (new) The method of claim 53, further comprising:

- (e) introducing a recombinase into the cell identified in step (d), wherein the endogenous gene locus flanked by the site-specific recombination sites is deleted.

55. (new) The method of claim 54, further comprising:

(f) creating a vector containing the site-specific recombination sites flanking a replacing gene locus; and

(g) introducing the vector of (f) into the cell of (e) such that, through recombination, the replacing gene locus is inserted between the site-specific recombination sites.

56. (new) The method of claim 54, further comprising:

(f) obtaining a large cloned genomic fragment containing, in whole or in part, a replacing region gene locus;

(g) using bacterial homologous recombination to genetically modify the cloned fragment of (f) to create a third LTVEC comprising the replacing region gene locus flanked by a downstream homology arm containing a region homologous to the 3' end of the endogenous gene locus region and an upstream homology arm containing a region homologous to the 5' end of the endogenous gene locus region; and

(h) introducing the third LTVEC of (g) into the cell of (e) to replace the deleted endogenous gene locus.

57. (new) The method of claim 53, wherein a site-specific recombination site is selected from one or more of loxP, lox511 and lox2272.

58. (new) The method of claim 54, wherein the recombinase is CRE.

59. (new) A modified endogenous gene locus produced by the method of claim 51, 52, 53, 55, or 56.

60. (new) The method of claim 53, 54, 55, or 56, wherein the eukaryotic cell is an embryonic stem cell.

61. (new) An embryonic stem cell produced by the method of claim 60.

62. (new) A transgenic non-human mouse generated from the embryonic stem cell of claim 61.

63. (new) The method of claim 51, 52, or 53, wherein the quantitative assay comprises quantitative PCR, FISH, comparative genomic hybridization, isothermic DNA amplification, or quantitative hybridization to an immobilized probe.

64. (new) The method of claim 63, wherein the quantitative PCR comprises TaqMan® technology or quantitative PCR using molecular beacons.

65. (new) A method of creating, in a eukaryotic cell, a modified endogenous immunoglobulin variable region gene locus flanked downstream by a site-specific recombination site comprising:

(a) creating a large targeting vector for use in eukaryotic cells (LTVEC) comprising a site-specific recombination site, a downstream homology arm containing a region homologous to a 3' end of the

endogenous immunoglobulin variable region gene locus region and an upstream homology arm within the locus;

(b) introducing the LTVEC of (a) into a eukaryotic cell; and

(c) using a quantitative assay to detect modification of allele (MOA) in the endogenous immunoglobulin variable region gene locus to identify those eukaryotic cells in (b) in which the endogenous immunoglobulin variable region gene locus is flanked downstream by the site-specific recombination site.

66. (new) A method of creating, in a eukaryotic cell, a modified endogenous immunoglobulin variable region gene locus flanked upstream by a site-specific recombination site comprising:

(a) creating a large targeting vector for use in eukaryotic cells (LTVEC) comprising a site-specific recombination site, an upstream homology arm containing a region homologous to the 5' end of the endogenous immunoglobulin variable region gene locus region and a downstream homology arm within the locus;

(b) introducing the LTVEC of (a) into the eukaryotic cell; and

(c) using a quantitative assay to detect modification of allele (MOA) in the endogenous immunoglobulin variable region gene locus to identify those eukaryotic cells in (b) in which the endogenous immunoglobulin variable region gene locus is flanked upstream by the site-specific recombination site.

67. (new) A method of creating, in a eukaryotic cell, a modified endogenous immunoglobulin variable region gene locus flanked by site-specific recombination sites comprising:

(a) creating a first large targeting vector for use in eukaryotic cells (LTVEC) comprising the site-specific recombination site, a downstream homology arm containing a region homologous to the 3' end of the endogenous immunoglobulin variable region gene locus region and an upstream homology arm within the locus;

(b) creating a second LTVEC comprising the site-specific recombination site, an upstream homology arm containing a region that flanks the 5' end of the endogenous immunoglobulin variable region gene locus region and a downstream homology arm within the locus;

(c) introducing the first and second LTVECs into the eukaryotic cell; and

(d) using a quantitative assay to detect modification of allele (MOA) in the endogenous immunoglobulin variable region gene locus to identify eukaryotic cells in which the site-specific recombination sites are flanking the endogenous immunoglobulin variable region gene locus.

68. (new) The method of claim 67, further comprising:

(e) introducing a recombinase into the cell identified in step (d), wherein the endogenous immunoglobulin variable region gene locus flanked by the site-specific recombination sites is deleted.

69. (new) The method of claim 68, further comprising:

(f) creating a vector containing the site-specific recombination sites flanking a human immunoglobulin variable region gene locus; and

(g) introducing the vector of (f) into the cell of (e) such that, through recombination, the human immunoglobulin variable region gene locus, in whole or in part, is inserted between the site-specific recombination sites.

70. (new) The method of claim 67, further comprising:

(f) obtaining a large cloned genomic fragment containing, in whole or in part, a human immunoglobulin variable region gene locus;

(g) using bacterial homologous recombination to genetically modify the cloned fragment of (f) to create a third LTVEC comprising the replacing region gene locus flanked by a downstream homology arm containing a region homologous to the 3' end of the endogenous gene locus region and an upstream homology arm containing a region homologous to the 5' end of the endogenous gene locus region; and

(h) introducing the third LTVEC of (g) into the cell of (e) to replace the deleted endogenous gene locus.

71. (new) The method of claim 67, 68, or 69, wherein the eukaryotic cell is a mouse embryonic stem cell.

72. (new) A mouse embryonic stem cell produced by the method of claim 71:

73. (new) A modified endogenous immunoglobulin variable region gene locus produced by the method of claim 65, 66, 67, or 69.

74. (new) A transgenic non-human mouse generated by introducing the embryonic stem cell of claim 72 into a blastocyst which is introduced into a surrogate mother for gestation.

75. (new) A method of replacing, in whole or in part, an endogenous immunoglobulin variable region gene locus with part or all of a human immunoglobulin variable region gene locus comprising:

(a) creating a first large targeting vector for use in eukaryotic cells (LTVEC) comprising a site-specific recombination site, a downstream homology arm containing the region immediately adjacent to, but not including, the J segments of the immunoglobulin variable gene locus region and an upstream homology arm within the variable gene locus;

(b) creating a second LTVEC comprising a site-specific recombination site, an upstream homology arm containing the region adjacent to the most distal V gene segment, but not containing any V gene segments of the immunoglobulin variable gene locus region and a downstream homology arm within the variable gene locus;

(c) introducing the first and second LTVECs into a eukaryotic cell;

(d) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify a cell in which the site-specific recombination sites flank the endogenous variable region gene locus;

(e) creating a vector containing the site-specific recombination sequences flanking all or part of a human immunoglobulin variable gene locus; and

(f) introducing the vector of (e) into the identified cell such that, through recombination, the endogenous immunoglobulin variable region gene locus is replaced, in whole or in part, with all or part of a human immunoglobulin variable gene locus gene locus.

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76. (new) The method of claim 74 , wherein the eukaryotic cell is a mouse embryonic stem cell.

77. (new) A mouse embryonic stem cell produced by the method of claim 76.

78. (new) A transgenic non-human mouse generated from the embryonic stem cell of claim 77.